Clinical spectrum and follow-up in six individuals with Lamb-Shaffer syndrome (SOX5)

Clinical spectrum and follow-up in six individuals with Lamb–Shaffer syndrome (SOX5)

Giovanni Innella[1], Donatella Greco[2], Diana Carli[3], Pamela Magini[1], Elisa Giorgio[4], Ornella Galesi[2], Giovanni Battista Ferrero[4], Corrado Romano[2], Alfredo Brusco[4,5], Claudio Graziano[1*]

1. Medical Genetics Unit, Policlinico di S. Orsola, University of Bologna, Bologna, Italy
2. Oasi Research Institute – IRCCS, Troina, Italy
3. Department of Public Health and Pediatric Sciences, University of Turin, 10126 Turin, Italy
4. Department of Medical Sciences, University of Turin, 10126 Turin, Italy
5. Unit of Medical Genetics, "Città della Salute e della Scienza" University Hospital, 10126 Turin, Italy

* Correspondence:

Dr Claudio Graziano, Medical Genetics Unit, Policlinico di S. Orsola, Alma Mater University of Bologna. Via Massarenti 9, 40138 Bologna, Italy

Phone +39 051 2143694; fax +39 051 2088416; e-mail: claudio.graziano@unibo.it

The authors declare no conflict of interest.

Key words: SOX5, Lamb–Shaffer syndrome, mosaicism, soxopathy

Running title: Lamb–Shaffer syndrome in adults

To the editor:

Lamb–Shaffer syndrome (LAMSHF) is a neurodevelopmental disorder caused by heterozygous loss of function genetic alterations in *SOX5*, which result in haploinsufficiency (Lamb et al., 2012; Zawerton et al., 2019a). *SOX5* maps on chromosome 12p12.1 and is part of a multigenic family encoding for transcription factors that contain a high-mobility-group (HMG) domain, like that of SRY protein (Schepers et al., 2002). It is now recognized that the SOX family plays pivotal roles in many developmental and pathological processes by governing cell type-specific genetic programs, which drive many crucial biological processes, including sex determination, neurogenesis, and skeletogenesis (Kamachi and Kondoh, 2013). In humans, pathogenic variants in several SOX genes were shown to cause developmental disorders (reviewed in Angelozzi and Lefebvre, 2019). Besides *SOX5*, specific neurodevelopmental diseases were recently associated with pathogenic variants in *SOX4* and *SOX11* (Hempel et al., 2016; Zawerton et al., 2019b).

Clinically, LAMSHF syndrome is mainly characterized by developmental delay, speech delay, intellectual disability, and behavioral disturbances, with other corroborating features such as ophthalmologic and skeletal abnormalities (Zawerton et al., 2019a). The clinical spectrum is broad, without clear genotype-phenotype correlations. Different types of *SOX5* alterations were described, including intragenic deletions, larger 12p12 deletions, reciprocal translocation with breakpoint within *SOX5*, truncating variants, and missense variants which clustered in the HMG domain (Lamb et al., 2012; Nesbitt et al., 2015; Fukushi et al., 2018; Zawerton et al., 2019a).

We report clinical and molecular data of six individuals from four families, carrying *SOX5* alterations. Written informed consent for genetic studies and for inclusion in the study was obtained from parents/guardians according to local ethic agreements. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Four subjects (Ia, Ib, II and III) harbored a *SOX5* deletion detected by chromosomal microarray analysis (SurePrint G3 ISCA v2 CGH 8x60K, Agilent Technologies) (Table 1). These deletions range in size from around 50 to 300 kb and involve different breakpoints; they are restricted to distinct segments of *SOX5* always including the HMG DNA-binding domain. The deletion identified in patient III was de novo, while for patient II we could only exclude maternal transmission; the father was reported healthy but he was not available for the test. qPCR analysis of patient Ic (father of Ia and Ib) suggested a possible mosaicism for the deletion, with copy number ratio between test and reference DNAs of about 0.8. The DNA of patient Ic was further analyzed through array-CGH: the average log2ratio value of the altered oligonucleotides in *SOX5* region allowed to estimate a 35% of cells with the deletion, according to the formula proposed by Valli et al. (2011). Only DNA from peripheral blood was tested. Finally, we found the c.1672C>T: p.Arg558Cys (NM_006940.6) heterozygous missense variant in patient IV by clinical exome sequencing (SOPHiA exome solution, 4,490 genes, February 2020), performed after chromosomal microarray analysis. The variant was *de novo* and absent in gnomAD (ver2.1.1). The arginine 558 is located in the HMG domain and affects a residue which is highly evolutionary conserved in *SOX5*, not only in vertebrate orthologs but also in distantly related species such as *D.melanogaster* and *C.elegans*, and in human SOX protein paralogs. The variant is reported in ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar; code VCV000806852.4, likely pathogenic). Furthermore, both *SRY* (p.Arg62Gly and p.Arg62Pro) and *SOX10* (p.Arg106Trp) variants in residues equivalent to the one affected in this subject were shown to cause disease (Affara et al., 1993; Buonocore et al., 2019; Chaoui et al., 2011). Overall, using ACMG criteria, we classified the c.1672C>T: p.Arg558Cys variant as likely pathogenic (Richards et al., 2015).

Clinical features of our cases are summarized in Table 1. Patients Ia and Ib are adult brothers with overlapping phenotype. Patient Ia was born at term after a pregnancy complicated by the threat of premature birth at the 6th month. Biometric measurements at birth and in the first year of life were

normal; afterwards he presented a growth delay. He suffered several episodes of febrile seizures up to the age of 3 years, and he undertook pharmacological therapy; EEG highlighted multifocal discharges in Rolandic, parietal and temporal regions, associated with frequent paroxysmal phenomena. Brain MRI did not reveal any anomalies. He showed a global developmental delay: autonomous walking was acquired after 5 years of age and he had a gait disturbance. Speech delay was severe and the language remained remarkably simple. He showed behavioral problems with episodes of aggression/temper tantrums; at the last examination (age 35 years), despite having reached primary autonomies, he presented severe intellectual disability with behavioral disturbances that worsened over time. His adult stature was 160 cm (-2.3 SD), well below his target height of 173 cm. Facial dysmorphic features also changed over the years (Figure 1): as a child, he had triangular face, thin nose and lips, narrow palate, prominent upper central incisors; at the last clinical evaluation, he showed relative microcephaly (52.2 cm, -2.0 SD), broad forehead, triangular face, strabismus, very narrow palate, gingival overgrowth with upper arch teeth protrusion, thin upper and full lower lip, prominent chin and protruding ears.

Patient Ib never came personally to our attention. The clinical course was reported as similar to his older brother. Pregnancy and birth were uneventful and he had no growth delay. According to medical records, he presented with absence-type epilepsy for which he undertook pharmacological therapy, with good control of seizures; EEG highlighted bi-central predominantly multifocal anomalies and widespread sleep-activated anomalies; brain MRI revealed areas of altered white matter intensity of uncertain interpretation. He had strabismus. Developmental delay was severe: autonomous walking was acquired at 19 months of age and broad-based gait and motor stereotypies were reported during childhood. Language remained poor, he showed severe behavioral problems which worsened over time with increasingly frequent outburst of aggression and, at the age of 30, was in an Institution for persons with intellectual disability. Facial features were evaluated thanks to the pictures provided by his family: when he was a child, he had a thin nose and upper lip, wide

open mouth, prominent upper central incisors and protruding ears; at the age of 30, he had microcephaly, high forehead, gingival overgrowth with upper arch teeth protrusion, prominent chin and protruding ears.

Their father (patient Ic), 65 years, was born at term after an uneventful pregnancy, main stages of psychomotor development were reached with mild delay; he dropped out of school when he was only 8 years old. He showed severe psychiatric problems; specifically, mild intellectual disability with dysthymic and personality disorder and behavioral problems were diagnosed. He never had seizures. Over the years, he was hospitalized several times for psychotic episodes and aggressive behavior. He left the family and was a homeless man for some years; currently, he is in the care of Social Services. At clinical evaluation, his height was normal (178 cm) and he had no significant facial dysmorphism.

Patient II, a 15-year-old girl from Romania, was born preterm (34^{th} week of gestation) with a birth weight of 2200 g. As a newborn, she was hospitalized due to poor growth and feeding difficulties for one month. Main stages of psychomotor development were reached with delay: autonomous walking was possible at the age of 2 years, sphincter control after 3 years; she presented with speech delay and subsequent evidence of language dyspraxia. At 3-4 years, she showed critical episodes of doubtful nature, characterized by labial cyanosis and impaired state of consciousness. She came to Italy at the age of 7 years. Mild intellectual disability, speech disorder and attention deficit hyperactivity disorder were diagnosed. She also showed behavioral problems characterized by motor restlessness, oppositional behavior, poor tolerance to frustrations and outbursts of aggression. EEG and brain-MRI did not reveal significant anomalies. She also showed mild hypotonia, hypermetropic astigmatism, scoliosis and hypothyroidism. At clinical evaluation, she had normal height and no significant facial dysmorphism.

Patient III, a 27-year-old man, was born at 36 weeks of gestation due to untimely rupture of the membranes and had a neonatal distress. He showed epilepsy for which he undertook pharmacological therapy; EEG highlighted short diffuse discharges of SW complexes, better represented on the right regions; brain-CT revealed mild asymmetry of the occipital horns. Main stages of psychomotor development have been reached with delay and at last examination he showed moderate intellectual disability. At clinical evaluation, he had short stature (160 cm), right-angle scoliosis, hypertrichosis and peculiar craniofacial features such as microcephaly, wide upper central incisors, low anterior hairline, bilateral ptosis, synophris, strabismus.

Patient IV, a 3-year-old child, was born at term after an unremarkable pregnancy. Biometric measurements at birth were normal and no neonatal issues were reported. He showed a global developmental delay: sitting position was acquired at 14 months, autonomous walking at 24 months, and the language at the last evaluation (3 years and 6 months) had not yet been acquired. Brain-MRI revealed thinning of corpus callosum. At the last clinical evaluation, he presented with a height of 101 cm (72^{th} centile, +0.57 SDS), weight of 17 Kg (80^{th} centile, +0.84 SDS), OFC 48 cm (10^{th} centile, -1.26 SDS), ear length 6 cm (>+2 SDS) and some peculiar craniofacial features such as bitemporal narrowing, strabismus, macrotia, tubular nose with bifid tip and micrognathia.

Limited information is often available on adult patients with rare disorders. We note that epilepsy is common in LAMSHF syndrome, but generally drug-responsive, and adult patients tend to be seizure-free. On the contrary, behavioral abnormalities (including an aggressive behavior) are common and worsen over time, often leading to a loss of independence. A timely social intervention is thus warranted. Facial dysmorphisms also evolve over time, as we observed in siblings Ia and Ib, for which we were able to collect photos from early childhood to adulthood (Figure 1): both had a triangular face as children, with thin nose and lips, large upper central incisors and protruding ears, while facial features tend to become coarser in time and they developed microcephaly with high and broad forehead, gingival overgrowth with upper arch teeth

protrusion, prominent chin. Our study also allowed the delineation of extra-neurological features, which are common but heterogeneous. Three patients had scoliosis and three had growth retardation, which is usually mild, although two of these patients had short stature in adulthood. Ophthalmological abnormalities are common (strabismus, pale papilla, hypermetropic astigmatism), as already reported.

The clinical spectrum of LAMSHF syndrome is wide, without clear genotype-phenotype correlation; the genetic background and/or environmental factors are likely to modulate the penetrance and degree of disease severity. Even patients with recurrent variants exhibited clinical variability, but this is not unexpected, given that haploinsufficiency is the accepted pathogenic mechanism. Patients with missense variants in the HMG-domain tended to have milder language deficits (Zawerton et al., 2019a), however, the number of affected individuals did not allow to draw firm conclusions. We report one single patient with an HMG-domain missense variant, who was non-verbal at the time of the last evaluation (three and a half years old). Further factors to consider in clinical variability are coinheritance of a distinct genetic defect (Quintela et al., 2015), and mosaicism, which is relatively frequent in LAMSHF syndrome (at least 14% in Zawerton et al., 2019a) and can be identified in unaffected parents or in patients with less penetrant disease. Most individuals with LAMSHF syndrome harbor *SOX5* de novo variants searched in blood, which does not exclude mosaicism in other tissues. It would be interesting to compare the clinical severity in children of mosaic parents versus patients with de novo variants, but a large patient cohort would be needed. Nevertheless, mosaicism is likely underestimated (Liu et al., 2020) and represents a big issue for genetic counseling and mental health as it is nicely illustrated in family 1 in the present study. Severe intellectual disability was present in two brothers born to apparently healthy parents; at closer inspection, the father was reported to suffer serious psychiatric health issues from childhood and was found to carry the *SOX5* deletion in a mosaic state. Improvement of genetic methodologies for the detection of mosaic variants will likely allow the identification of mutations

causing seemingly sporadic mental disorders, which could be limited to brain (Yourov et al., 2018), or involve the germinal line with a risk of transmission (Breuss et al., 2020).

In conclusion, LAMSHF syndrome is a rare and recently described condition which, however, will likely be diagnosed with increasing frequency thanks to the improvement of molecular analysis techniques and their widespread application to patients with neurodevelopmental disorders. Many deletions are small and involve *SOX5* only. Clinical relevance can still be difficult to define for small copy number variants (Magini et al., 2019), and we note that the deletion in patient III was identified before *SOX5* was established as a gene associated to neurodevelopmental disorder, and was initially classified as likely benign. At the same time, the spectrum and relevance of *SOX5* variants, especially if they fall outside the HMG domain, is still uncertain. The description of new patients is fundamental to increase our knowledge of these disorders, to precisely define molecular characteristics and clinical phenotype, the evolution over time, comorbidities, and possible genotype-phenotype correlations.

Acknowledgements

We are grateful to the patients and their families for their precious collaboration. This research received funding specifically appointed to Department of Medical Sciences, University of Turin, from the Italian Ministry for Education, University and Research (Ministero dell'Istruzione, dell'Università e della Ricerca - MIUR) under the programme "Dipartimenti di Eccellenza 2018 – 2022" Project code D15D18000410001. DG, OG, and CR received funding from the Italian Ministry of Health (Project RC n. 2757328).

Author contribution:

Giovanni Innella and Claudio Graziano: conceptualization, clinical evaluation, data acquisition and analysis, manuscript preparation and revisions. Donatella Greco, Diana Carli, Ornella Galesi,

Giovanni Battista Ferrero and Corrado Romano: clinical evaluation, data acquisition and analysis. Pamela Magini, Alfredo Brusco and Elisa Giorgio: molecular analysis and results' interpretation. All authors read and approved the final manuscript prior to submission.

References

Affara, N.A., Chalmers I.J., & Ferguson-Smith, M.A. (1993). Analysis of the SRY gene in 22 sex-reversed XY females identifies four new point mutations in the conserved DNA binding domain. *Human Molecular Genetics*, 2, 785-789. doi: 10.1093/hmg/2.6.785

Angelozzi, M., & Lefebvre, V. (2019). SOXopathies: growing family of developmental disorders due to SOX mutations. *Trends in Genetics*, 35, 658–671. doi: 10.1016/j.tig.2019.06.003

Breuss, M.W., Antaki, D., George, R.D., Kleiber, M., James, K.N., Ball, L.L., ... Gleeson, J.G. (2020). Autism risk in offspring can be assessed through quantification of male sperm mosaicism. *Nature Medicine*, 26, 143-150. doi:10.1038/s41591-019-0711-0

Buonocore, F., Clifford-Mobley, O., King, T.F.J., Striglioni, N., Man, E., Suntharalingham, J.P., … Achermann, J.C. (2019). Next-Generation Sequencing Reveals Novel Genetic Variants (SRY, DMRT1, NR5A1, DHH, DHX37) in Adults With 46,XY DSD. *Journal of the Endocrine Society*, 3, 2341–2360. doi:10.1210/js.2019-00306

Chaoui, A., Watanabe, Y., Touraine, R., Baral, V., Goossens, M., Pingault, V., Bondurand, N. (2011). Identification and functional analysis of SOX10 missense mutations in different subtypes of Waardenburg syndrome. *Human Mutation*, 32, 1436-1449. doi:10.1002/humu.21583

Fukushi, D., Yamada, K., Suzuki, K., Inaba, M., Nomura, N., Suzuki, Y., ... Wakamatsu N. (2018). Clinical and genetic characterization of a patient with SOX5 haploinsufficiency caused by a de novo balanced reciprocal translocation. *Gene*, 655, 65–70. doi: 10.1016/j.gene.2018.02.049

Hempel, A., Pagnamenta, A.T., Blyth, M., Mansour, S., McConnell, V., Kou, I., ... McNeill, A. (2016). Deletions and de novo mutations of SOX11 are associated with a neurodevelopmental disorder with features of Coffin-Siris syndrome. *Journal of Medical Genetics*, 53, 152–162. doi: 10.1136/jmedgenet-2015-103393

Kamachi, Y., & Kondoh, H. (2013) Sox proteins: regulators of cell fate specification and differentiation. *Development*, 140, 4129–4144. doi: 10.1242/dev.091793

Lamb, A.N., Rosenfeld, J.A., Neill, N.J., Talkowski, M.E., Blumenthal, I., Girirajan, S., … Shaffer, L.G. (2012). Haploinsufficiency of SOX5 at 12p12.1 is associated with developmental delays with prominent language delay, behavior problems, and mild dysmorphic features. *Human Mutation*, 33, 728–740. doi: 10.1002/humu.22037

Liu, Q., Karolak, J.A., Grochowski, C.M., Wilson, T.A., Rosenfeld, J.A., Bacino, C.A., ... Stankiewicz, P. (2020). Parental somatic mosaicism for CNV deletions - A need for more sensitive and precise detection methods in clinical diagnostics settings. *Genomics*, 112, 2937-2941. doi:10.1016/j.ygeno.2020.05.003

Magini, P., Scarano, E., Donati, I., Sensi, A., Mazzanti, L., Perri, A., ... Graziano, C. (2019). Challenges in the clinical interpretation of small de novo copy number variants in neurodevelopmental disorders. *Gene*, 706, 162–171. doi:10.1016/j.gene.2019.05.007

Nesbitt, A., Bhoj, E.J., McDonald Gibson, K., Yu, Z., Denenberg, E., Sarmady, M., … Santani, A. (2015). Exome sequencing expands the mechanism of SOX5-associated intellectual disability: A case presentation with review of sox-related disorders. *American Journal of Medical Genetics Part A*, 167A, 2548–2554. doi:10.1002/ajmg.a.37221

Quintela, I., Barros, F., Lago-Leston, R., Castro-Gago, M., Carracedo, A., Eiris, J. (2015). A maternally inherited 16p13.11-p12.3 duplication concomitant with a de novo SOX5 deletion in a

male patient with global developmental delay, disruptive and obsessive behaviors and minor dysmorphic features. *American Journal of Medical Genetics Part A*, 167, 1315-1322. doi:10.1002/ajmg.a.36909

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., ... Rehm, H.L. (2015). Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, 17, 405–424. doi:10.1038/gim.2015.30

Schepers, G.E., Teasdale, R.D., Koopman, P. (2002) Twenty pairs of sox: extent, homology, and nomenclature of the mouse and human sox transcription factor gene families. *Developmental Cell*, 3, 167–170. doi:10.1016/S1534-5807(02)00223-X

Valli, R., Maserati, E., Marletta, C., Pressato, B., Lo Curto, F., Pasquali, F. (2011). Evaluating chromosomal mosaicism by array comparative genomic hybridization in hematological malignancies: the proposal of a formula. *Cancer Genetics*,204, 216-218. doi:10.1016/j.cancergen.2011.02.002

Yurov, Y.B., Vorsanova, S.G., Demidova, I.A., Kolotii, A.D., Soloviev, I.V., Iourov, I.Y. (2018). Mosaic Brain Aneuploidy in Mental Illnesses: An Association of Low-level Post-zygotic Aneuploidy with Schizophrenia and Comorbid Psychiatric Disorders. *Current Genomics*, 19, 163-172. doi:10.2174/1389202918666170717154340

Zawerton, A., Mignot, C., Sigafoos, A., Blackburn, P.R., Haseeb, A., McWalter, K., … Depienne, C. (2019). Widening of the genetic and clinical spectrum of Lamb–Shaffer syndrome, a neurodevelopmental disorder due to SOX5 haploinsufficiency. *Genetics in Medicine*, 22, 524-537. doi: 10.1038/s41436-019-0657-0

Zawerton, A., Yao, B., Yeager, J.P., Pippucci, T., Haseeb, A., Smith, J.D., ... Lefebvre, V. (2019). De novo SOX4 variants cause a neurodevelopmental disease associated with mild dysmorphism. *American Journal of Human Genetics*, 104, 246–259. doi: 10.1016/j.ajhg.2018.12.014

TABLE 1 Genotype, phenotypic and demographic information for individuals I-IV

	Sex	Ethnic origin	Age at last examination (yrs)	Variant type	Variant coordinates (hg19)	Inheritance	ID (level)	Behavioral abnormalities	Seizures	Other neurologic features	Brain findings	Dysmorphic features	Ocular features	Skeletal features	Other
Ia	M	Ita	35	Del	chr12:23,589,160-23,738,179	Paternal mosaic	Yes (severe)	Yes	Yes	Gait disturbance	None	Microcephaly, triangular face, broad forehead, protruding ears, high and narrow palate, large upper central incisors	Strabismus	Scoliosis	Mitral valve prolapse, short stature
Ib	M	Ita	(30)	Del	chr12:23,589,160-23,738,179	Paternal mosaic	Yes (severe)	Yes	Yes	Gait disturbance	Areas of altered white matter intensity	Wide open mouth, large upper central incisors, protruding ears	Strabismus	No	No
Ic	M	Ita	65	Del	chr12:23,589,160-23,738,179	Mosaic	Yes (mild)	Yes	No	No	NP	No	No	No	No
II	F	Rom	15	Del	chr12:23,685,603-23,738,179	UK (mother not deleted)	Yes (mild)	Yes	Yes	Hypotonia	None	No	Hypermetropic astigmatism	Scoliosis	Hypothyroidism, growth delay
III	M	Ita	27	Del	chr12:23,685,603-23,979,812	DN	Yes (moderate)	No	Yes	No	Mild asimmetry of occipital horns	Microcephaly, wide upper central incisors, low anterior hairline, bilateral ptosis, synophris	Strabismus, pale papilla	Scoliosis	Short stature, hypertrichosis
IV	M	Ita	3	Mis	c.1672C>T (p.Arg558Cys)	DN	Yes (NA)	No	No	No	Thinning of corpus callosum	Microcephaly, bitemporal narrowing, macrotia, tubular nose with bifid tip, micrognathia	Strabismus	No	No

Abbreviations: M: male; F: female; Ita: Italian; Rom: Romanian; Del: deletion; Mis: missense; ID: intellectual disability; UK: unknown; DN: *de novo*; NP: not performed; NA: not available

Figure 1. Progression of facial phenotype in patient IA (upper row) and IB (lower row) from childhood to adulthood [Color figure can be viewed at wileyonlinelibrary.com]